Figure 7 in A shows the nucleotide sequence (SEQ ID NO: 4) of a AP-2 binding site, indicating three overlapping polyamide target sites, and in B, the schematic structure of five polyamides **(9-13)** that were designed to bind to the nucleotide sequences of these target sites 1-3.

REMARKS

The application has been amended herein to refer to updated sequence identifiers in conjunction with Applicant's response to the Notice to Comply mailed May 16, 2001. The replacement paragraphs to the specification provided herein incorporate the updated sequence identifiers, as appropriate. Marked-up copies of the original pages of the application bearing these modified paragraphs reflect the amendments in red ink. The sequence listing and CRF therefor are enclosed. The amendments to the application add no new matter.

In connection with the Sequence Listing submitted concurrently herewith, the undersigned hereby states that:

1. the content of the attached paper copy and the enclosed computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same; and

2. the submission, filed herewith in accordance with 37 C.F.R. § 1.821(g), does not include new matter.

Respectfully submitted,

Date: November 21, 2001

MICHAEL A. WHITTAKER
Reg. No. 46,230

Foley & Lardner
CUSTOMER NO. 23620
P.O. BOX 80278
SAN DIEGO, CA 92138-0278
Phone: (858) 847-6721
Fax: (858) 792-6773

Another approach utilizes cell-permeable small molecules that target particular DNA sequences. These molecules would be useful for the regulation of gene expression. The design of small synthetic DNA-binding ligands that recognize specific sequences in the DNA double helix has been a long standing goal of chemistry. Oligodeoxynucleotides that recognize the major groove of double-helical DNA via triple-helix formation bind to a broad range of sequences with high affinity and specificity. Although oligonucleotides and their analogs have been shown to interfere with gene expression, the triple helix approach is limited to purine tracks and suffers from poor cellular uptake.

Other small molecules have also been of interest as DNA-binding ligands. Wade, *et al.* reported the design of peptides that bind in the minor groove of DNA at 5'-(A,T)G(A,T)C(A,T)-3' sequences by a dimeric side-by-side motif (*J. Am. Chem. Soc.* 114, 8783-8794 (1992)). Mrksich, *et al.* reported antiparallel side-by-side motif for sequence specific-recognition in the minor groove of DNA by the designed peptide 1-methylimidazole-2-carboxamidenetropsin (*Proc. Natl. Acad. Sci. USA* 89, 7586-7590 (1992)). Pelton, J.G. & Wemmer, D.E. reported the structural characterization of a 2-1 distamycin A-d(CGCAAATTTGGC) complex by two-dimensional NMR (*Proc. Natl. Acad. Sci. USA* 86, 5723-5727 (1989)). (SEQ ID NO:5)

Dervan and colleagues have shown that synthetic pyrrole-imidizole polyamides bind DNA with excellent specificity and very high affinities, even exceeding the affinities of many sequence-specific transcription factors (Trauger, *et al.*, *Nature* 382, 559-561 (1996)). They further describe the recognition of DNA by designed ligands at subnanomolar concentrations. DNA recognition depends on side-by-side amino acid pairing of imidizole-pyrrole or pyrrole-pyrrole pairs in the minor groove. White, S., *et al.*, (1996) reported the effects of the A•T/T•A degeneracy of pyrrole-imidazole polyamide recognition in the minor groove of DNA (*Biochemistry* 35, 6147-6152 (1996)). White, *et al.* (1997) reported pairing rules for recognition in the minor groove of DNA by pyrrole-imidazole polyamides (*Chem. & Biol.* 4, 569-578), and demonstrated the 5'-3' N-C orientation preference for polyamide binding in the minor groove. Thus, polyamide molecules thus have the potential to act as inhibitors of protein-DNA interactions in the minor groove.

Suitable polyamides most preferably have a binding affinity at the dsDNA target sequence of at least 10^9 M^{-1} and a selectivity of at least about two. Selectivity is defined as the ratio of the binding affinity for the identified dsDNA target sequence to the binding affinity for a single base-pair mismatch dsDNA sequence. In preferred embodiments, selectivity against at least 90% of single base mismatch sequences is greater than about 10.

In a related aspect of the present invention, compositions are provided that comprise a pharmaceutically acceptable excipient and a transcription-inhibiting amount of at least one polyamide of the invention. Each polyamide contains at least three complementary pairs of aromatic carboxamide residues, which pairs are selected to correspond to an identified nucleotide sequence of a dsDNA target. Preferably, the polyamides additionally comprise at least two aliphatic amino acid residues chosen from the group consisting of glycine, β-alanine, γ-aminobutyric acid, R-2,4-diaminobutyric acid, and 5-aminovaleric acid, and at least one terminal alkylamino residue, the polyamide having a binding affinity at the target dsDNA sequence of at least 10^9 M^{-1} and a selectivity of at least about two, selectivity being defined as the ratio of the binding affinity for the identified target dsDNA sequence to the binding affinity for a single base-pair mismatch dsDNA sequence.

The invention further provides methods suitable for treating a subject having a condition associated with abnormal expression of a cellular oncogene. The subject is preferably a human patient and, more particularly, one afflicted with breast cancer or other diseases or conditions associated with aberrant Her-2/neu oncogene expression.

Brief Description of the Drawings

Figure 1 depicts the HER2/neu promoter, showing the nucleotide sequence (SEQ ID NO: 1) in A, including binding sites of ESX, AP-2, OB2-1, and TBP ("TATA") transcription factors, and in B, a schematic diagram, not to scale, showing the ESX, TBP, and OB2-1 binding sites.

Figure 2 in A shows the nucleotide sequence (SEQ ID NO: 2) of the ESX binding site, indicating four overlapping polyamide target sites, and in B, the schematic structure of eight

polyamides (1-8) that have been designed to bind to the nucleotide sequences of these target sites 1-4, in which N-methylimidazole carboxamides are represented by filled circles, N-methylpyrrole carboxamides by empty circles, and β-alanine amino acids by unfilled diamonds. 2, 4-diaminobutyric acid is represented by a curved line bearing an amino group and the N,N-dimethylaminopropyl substituent (and the C-terminus) by a positively charged half-circle.

Figure 3 illustrates a typical solid-phase synthesis scheme, using polyamide 1 as an example.

Figure 4 shows the structural formulas of eight polyamides that have been designed to bind to the ESX binding site, as well as MALDI-MS data that characterize each compound.

Figure 5 is a graphical representation of the results of a DNase I footprint titration of polyamide 1 on the 188 base-pair 5'-end-labeled DNA fragment, showing in A an autoradiogram: lane 1, A reaction; lane 2 to 12, 20 pM, 40 pM, 80 pM, 100 pM, 200 pM, 400 pM, 1 nM, 2 nM, 4 nM 10 nM, 20 nM polyamide 1, all reactions containing 15 kcpm DNA fragment, 10 mM Tris HCl (pH 7.0), 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$, the match- and the single base-pair mismatch site shown to the right side of the autoradiogram, and in B, the schematic structure of polyamide 1.

(SEQ ID NO: 3)

Figure 6 summarizes the results of DNase I footprint titrations, showing in A, a portion of the nucleotide sequence of the HER2/neu promoter containing the ESX binding site with polyamide binding sites 1-4 indicated, and in B, the four polyamide target sites with corresponding schematic representations of polyamides 1-8.

(SEQ ID NO: 4)

Figure 7 in A shows the nucleotide sequence of a AP-2 binding site, indicating three overlapping polyamide target sites, and in B, the schematic structure of five polyamides (9-13) that were designed to bind to the nucleotide sequences of these target sites 1-3.